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Application No. 10/811,138

### AMENDMENT TO THE CLAIMS

*A listing of the claims pending in this patent application are set forth below.*

**Claim 1 (withdrawn):** An isolated nucleic acid capable of hybridizing to SEQ ID NO: 3 under stringent conditions and encoding a protein which is capable of binding coelenterazine and molecular oxygen and emitting light, said protein having an isoleucine residue in a first position corresponding to position 132 of SEQ ID NO: 4.

**Claim 2 (withdrawn):** An isolated nucleic acid capable of hybridizing to SEQ ID NO: 3 under stringent conditions and encoding a protein which is capable of binding coelenterazine and molecular oxygen and emitting light, wherein a non-natural amino acid is incorporated into a position corresponding to 132 of SEQ ID NO: 4, during translation of said protein.

**Claim 3 (withdrawn):** An isolated nucleic acid capable of hybridizing to SEQ ID NO: 3 under stringent conditions and encoding a protein which is capable of binding coelenterazine and molecular oxygen and emitting light, said protein having a cysteine residue in a first position corresponding to position 69 of SEQ ID NO: 4.

**Claim 4 (withdrawn):** An isolated nucleic acid capable of hybridizing to SEQ ID NO: 3 under stringent conditions and encoding a protein which is capable of binding coelenterazine and molecular oxygen and emitting light, said protein having a cysteine residue in a first position corresponding to position 70 of SEQ ID NO: 4.

**Claim 5 (withdrawn):** An isolated nucleic acid capable of hybridizing to SEQ ID NO: 3 under stringent conditions and encoding a protein which is capable of binding coelenterazine and molecular oxygen and emitting light, said protein having a cysteine residue in a first position corresponding to position 74 of SEQ ID NO: 4.

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**Claim 6 (withdrawn):** An isolated nucleic acid capable of hybridizing to SEQ ID NO: 3 under stringent conditions and encoding a protein which is capable of binding coelenterazine and molecular oxygen and emitting light, said protein having a cysteine residue in a first position corresponding to position 76 of SEQ ID NO: 4.

**Claim 7 (withdrawn):** An isolated nucleic acid capable of hybridizing to SEQ ID NO: 3 under stringent conditions and encoding a protein which is capable of binding coelenterazine and molecular oxygen and emitting light, said protein having a phenylalanine residue in a first position corresponding to position 132 of SEQ ID NO: 4.

**Claim 8 (withdrawn):** An isolated nucleic acid capable of hybridizing to SEQ ID NO: 3 under stringent conditions and encoding a protein which is capable of binding coelenterazine and molecular oxygen and emitting light, said protein having an tyrosine residue in a first position corresponding to position 86 of SEQ ID NO: 4.

**Claim 9 (withdrawn):** An isolated nucleic acid capable of hybridizing to SEQ ID NO: 3 under stringent conditions and encoding a protein which is capable of binding coelenterazine and molecular oxygen and emitting light, said protein having a cysteine residue in a first position corresponding to position 66 of SEQ ID NO: 4.

**Claim 10 (withdrawn):** An isolated nucleic acid capable of hybridizing to SEQ ID NO: 3 under stringent conditions and encoding a protein which is capable of binding coelenterazine and molecular oxygen and emitting light, said protein having a cysteine residue in a first position corresponding to position 65 of SEQ ID NO: 4.

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**Claim 11 (withdrawn):** An isolated nucleic acid capable of hybridizing to SEQ ID NO: 3 under stringent conditions and encoding a protein which is capable of binding coelenterazine and molecular oxygen and emitting light, said protein having a tyrosine residue in a first position corresponding to position 16 of SEQ ID NO: 4.

**Claim 12 (currently amended):** An isolated nucleic acid having at least 95% sequence identity similarity to SEQ ID NO: 3 ~~under stringent conditions and encoding wherein said nucleic acid encodes~~ a protein ~~which is capable of binding~~ encodes a protein that binds coelenterazine and molecular oxygen and ~~emitting~~ emits light, said protein having a tryptophan residue in a first position corresponding to position 82 of SEQ ID NO: 4.

**Claim 13 (currently amended):** An isolated nucleic acid having at least 95% sequence identity similarity to SEQ ID NO: 3 ~~under stringent conditions and encoding wherein said nucleic acid encodes~~ a protein ~~which is capable of binding~~ encodes a protein that binds coelenterazine and molecular oxygen and emitting light, said protein having a phenylalanine residue in a first position corresponding to position 82 of SEQ ID NO: 4.

**Claim 14 (withdrawn):** A kit comprising the protein of claim 1 and a coelenterazine selected from the group consisting of CTZ i, ip, h, hcp, cp, fcp, f, n, and native coelenterazine.

**Claim 15 (withdrawn):** A kit comprising the protein of claim 2 and a coelenterazine selected from the group consisting of CTZ i, ip, h, hcp, cp, fcp, f, n, and native coelenterazine.

**Claim 16 (withdrawn):** A kit comprising the protein of claim 3 and a coelenterazine selected from the group consisting of CTZ i, ip, h, hcp, cp, fcp, f, n, and native coelenterazine.

**Claim 17 (withdrawn):** A kit comprising the protein of claim 4 and a coelenterazine selected from the group consisting of CTZ i, ip, h, hcp, cp, fcp, f, n, and native coelenterazine.

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**Claim 18 (withdrawn):** A kit comprising the protein of claim 5 and a coelenterazine selected from the group consisting of CTZ i, ip, h, hcp, cp, fcp, f, n, and native coelenterazine.

**Claim 19 (withdrawn):** A kit comprising the protein of claim 6 and a coelenterazine selected from the group consisting of CTZ i, ip, h, hcp, cp, fcp, f, n, and native coelenterazine.

**Claim 20 (withdrawn):** A kit comprising the protein of claim 7 and a coelenterazine selected from the group consisting of CTZ i, ip, h, hcp, cp, fcp, f, n, and native coelenterazine.

**Claim 21 (withdrawn):** A kit comprising the protein of claim 8 and a coelenterazine selected from the group consisting of CTZ i, ip, h, hcp, cp, fcp, f, n, and native coelenterazine.

**Claim 22 (withdrawn):** A kit comprising the protein of claim 9 and a coelenterazine selected from the group consisting of CTZ i, ip, h, hcp, cp, fcp, f, n, and native coelenterazine.

**Claim 23 (withdrawn):** A kit comprising the protein of claim 1 and a coelenterazine selected from the group consisting of CTZ i, ip, h, hcp, cp, fcp, f, n, and native coelenterazine.

**Claim 24 (withdrawn):** A kit comprising the protein of claim 10 and a coelenterazine selected from the group consisting of CTZ i, ip, h, hcp, cp, fcp, f, n, and native coelenterazine.

**Claim 25 (withdrawn):** A kit comprising the protein of claim 11 and a coelenterazine selected from the group consisting of CTZ i, ip, h, hcp, cp, fcp, f, n, and native coelenterazine.

**Claim 26 (withdrawn):** A kit comprising the protein of claim 12 and a coelenterazine selected from the group consisting of CTZ i, ip, h, hcp, cp, fcp, f, n, and native coelenterazine.

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**Claim 27 (withdrawn):** A kit comprising the protein of claim 13 and a coelenterazine selected from the group consisting of CTZ i, ip, h, hep, cp, fcp, f, n, and native coelenterazine.

**Claim 28 (withdrawn):** An aequorin mutant protein encoded by the nucleic acid of claim 3 wherein the protein is conjugated to a fluorphore.

**Claim 29 (withdrawn):** The aequorin mutant of claim 28 wherein the fluorphore is IANBD ester.

**Claim 30 (withdrawn):** An aequorin mutant protein encoded by the nucleic acid of claim 4 wherein the protein is conjugated to a fluorphore.

**Claim 31 (withdrawn):** The aequorin mutant of claim 30 wherein the fluorphore is IANBD ester.

**Claim 32 (withdrawn):** An aequorin mutant protein encoded by the nucleic acid of claim 5 wherein the protein is conjugated to a fluorphore.

**Claim 33 (withdrawn):** The aequorin mutant of claim 32 wherein the fluorphore is IANBD ester.

**Claim 34 (withdrawn):** An aequorin mutant protein encoded by the nucleic acid of claim 6 wherein the protein is conjugated to a fluorphore.

**Claim 35 (withdrawn):** The aequorin mutant of claim 34 wherein the fluorphore is IANBD ester.

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**Claim 36 (withdrawn):** The nucleic acid of claim 2 wherein the non natural amino acid is fluorotyrosine or fluorotryptophan.

**Claim 37 (withdrawn):** The nucleic acid of claim 36 wherein the fluorotyrosine is 3-fluoro-l-tyrosine.

**Claim 38 (withdrawn):** The nucleic acid of claim 2 wherein the non natural amino is 5-fluoro-l-tryptophan.

**Claim 39 (withdrawn):** A method of identifying inhibitors of bond-breaking enzymes comprising:

- (a) immobilizing a fusion protein encoded by a fusion protein nucleic acid comprising:
  - (1) any one of the nucleic acids of claims 1 to 13;
  - (2) operably linked to a second nucleic acid encoding a bond-breaking enzyme recognition site;in a first locus and a second locus;
- (b) contacting said fusion protein with a candidate compound in the presence of the bond-breaking enzyme in said first locus;
- (c) contacting said fusion protein with the bond-breaking enzyme in said second locus; and
- (d) determining whether there is an increase in the intensity of light emission at said first locus relative to light emission in said second locus.

**Claim 40 (withdrawn):** A method of identifying inhibitors of HIV-1 protease comprising:

- (a) immobilizing a fusion protein encoded by a fusion protein nucleic acid comprising:

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- (1) an isolated nucleic acid capable of hybridizing to SEQ ID NO: 3 under stringent conditions and encoding a protein which is capable of binding coelenterazine and molecular oxygen and emitting light, said protein having one or two amino acid substitutions selected from the group consisting of, an isoleucine residue in a position corresponding to position 132 of SEQ ID NO: 4, a non-natural amino acid incorporated into a position corresponding to 132 of SEQ ID NO: 4, a cysteine residue in a position corresponding to position 69 of SEQ ID NO: 4; a cysteine residue in a position corresponding to position 70 of SEQ ID NO: 4, a cysteine residue in a position corresponding to position 74 of SEQ ID NO: 4, a cysteine residue in a position corresponding to position 76 of SEQ ID NO: 4, a phenylalanine residue in a position corresponding to position 132 of SEQ ID NO: 4, a tyrosine residue in a position corresponding to position 86 of SEQ ID NO: 4, a cysteine residue in a position corresponding to position 66 of SEQ ID NO: 4, a cysteine residue in a position corresponding to position 65 of SEQ ID NO: 4, a tyrosine residue in a position corresponding to position 16 of SEQ ID NO: 4, a tryptophan residue in a position corresponding to position 82 of SEQ ID NO: 4, and a phenylalanine residue in a position corresponding to position 82 of SEQ ID NO: 4;
- (2) operably linked to a second nucleic acid encoding an HIV-1 enzyme recognition site;  
in a first locus and a second locus;
- (b) contacting said fusion protein with a candidate compound in the presence of the bond-breaking enzyme in said first locus;
- (c) contacting said fusion protein with the bond-breaking enzyme in said second locus; and

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- (d) determining whether there is an increase in the intensity of light emission at said first locus relative to light emission in said second locus.

**Claim 41 (withdrawn):** The method of claim 40 wherein the recognition site is Ser-Glu-Asn-Tyr-Pro-Ile-Val (SEQ ID NO: 5).

**Claim 42 (withdrawn):** The method of claim 40 wherein the fusion protein is conjugated to a fluorophore.

**Claim 43 (withdrawn):** The method of claim 40 wherein the fusion protein comprises a non-natural amino acid.

**Claim 44 (withdrawn):** The method of claim 43 wherein the non-natural amino acid is fluorotyrosine and is at a position corresponding to 132 of SEQ ID NO: 4.

**Claim 45 (withdrawn):** The method of claim 40 wherein the nucleic acid capable of hybridizing to SEQ ID NO: 3 under stringent conditions is any of the nucleic acids recited in claims 1 to 13.

**Claim 46 (withdrawn):** An isolated nucleic acid capable of hybridizing to SEQ ID NO: 5 under stringent conditions and encoding a protein which is capable of binding coelenterazine and molecular oxygen and emitting light, said protein having an serine residue in a first position corresponding to position 51, and a serine residue in a second position corresponding to position 75 of SEQ ID NO: 6.

**Claim 47 (withdrawn):** An isolated nucleic acid capable of hybridizing to SEQ ID NO: 5 under stringent conditions and encoding a protein which is capable of binding coelenterazine and molecular oxygen and emitting light, said protein having an serine residue in a first position



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corresponding to position 67, and a serine residue in a second position corresponding to position 75 of SEQ ID NO: 6.

**Claim 48 (withdrawn):** An isolated nucleic acid capable of hybridizing to SEQ ID NO: 5 under stringent conditions and encoding a protein which is capable of binding coelenterazine and molecular oxygen and emitting light, said protein having an serine residue in a first position corresponding to position 151 of SEQ ID NO: 6.

**Claim 49 (withdrawn):** A kit comprising the protein of claim 46 and a coelenterazine selected from the group consisting of CTZ i, ip, h, hcp, cp, fcp, f, n, and native coelenterazine.

**Claim 50 (withdrawn):** A kit comprising the protein of claim 47 and a coelenterazine selected from the group consisting of CTZ i, ip, h, hcp, cp, fcp, f, n, and native coelenterazine.

**Claim 51 (withdrawn):** A kit comprising the protein of claim 48 and a coelenterazine selected from the group consisting of CTZ i, ip, h, hcp, cp, fcp, f, n, and native coelenterazine.

**Claim 52 (withdrawn):** A method of identifying inhibitors of bond-breaking enzymes comprising:

- (a) immobilizing a fusion protein encoded by a fusion protein nucleic acid comprising:
  - (1) any one of the nucleic acids of claims 46 to 48;
  - (2) operably linked to a second nucleic acid encoding a bond-breaking enzyme recognition site;in a first locus and a second locus;
- (b) contacting said fusion protein with a candidate compound in the presence of the bond-breaking enzyme in said first locus;

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- (c) contacting said fusion protein with the bond-breaking enzyme in said second locus; and
- (d) determining whether there is an increase in the intensity of light emission at said first locus relative to light emission in said second locus.

**Claim 53 (withdrawn):** A method of identifying inhibitors of HIV-1 protease comprising:

- (a) immobilizing a fusion protein encoded by a fusion protein nucleic acid comprising:
  - (1) any one of the nucleic acids of claims 46 to 48;
  - (2) operably linked to a second nucleic acid encoding an HIV-1 enzyme recognition site;  
in a first locus and a second locus;
- (b) contacting said fusion protein with a candidate compound in the presence of the bond-breaking enzyme in said first locus;
- (c) contacting said fusion protein with the bond-breaking enzyme in said second locus; and
- (d) determining whether there is an increase in the intensity of light emission at said first locus relative to light emission in said second locus.